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Pendolmycin, a New Tumor Promoter of the Teleocidin A Class on Skin of CD-1 Mice

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Pendolmycin, isolated from *Nocardiopsis*, is a compound structurally similar to teleocidin A, one of the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-type tumor promoters. Pendolmycin has a C_5 dimethyl allyl group attached to C-7 of (−)-indolactam-V, whereas teleocidin A has a C_{10} linalyl group attached to the molecule. The structure-activity relationships of a hydrophobic moiety attached to (−)-indolactam-V were studied in four compounds, (−)-indolactam-V, pendolmycin, teleocidin A and newly synthesized 7-(nerolidyl)-(−)-indolactam-V in tests on inhibition of the specific [^{3}H]TPA binding to a particulate fraction of mouse skin, activation of protein kinase C and induction of both adhesion of HL-60 cells and ornithine decarboxylase in mouse skin. The potencies of the compounds for these activities increased mainly depending on the length of the hydrophobic group. Pendolmycin had a tumor-promoting activity on mouse skin initiated with a single application of 7,12-dimethylbenz[*a*]anthracene, and its potency was just between those of (−)-indolactam-V and teleocidin A. The role of the hydrophobic moiety is discussed with particular emphasis on the results obtained with 7-(nerolidyl)-(−)-indolactam-V.

Key words: Pendolmycin — Teleocidin A — 7-(Nerolidyl)-(−)-indolactam-V — Tumor promoter

Pendolmycin is a new indole alkaloid isolated from a strain of *Nocardiopsis*,[1] and is structurally similar to teleocidins A-1 and A-2[2] (Fig. 1). Since pendolmycin induces the same biochemical and biological activities as do two teleocidin A isomers,[3] it was expected to be a tumor promoter on mouse skin initiated with 7,12-dimethylbenz[*a*]anthracene (DMBA), as are the two teleocidin A isomers and 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Pendolmycin is made up of a C_5 dimethyl allyl group attached to the (−)-indolactam-V molecule (Fig. 1), which is the common structure of the two teleocidin A isomers and four teleocidin B isomers. (−)-Indolactam-V has two constituents, L-tryptophan and L-valine, which form a nine-membered lactam ring (providing the suffix V).[4] We previously reported that a large hydrophobic domain attached to the (−)-indolactam-V molecule is important for induction of biological and tumor-promoting activities.[5] Since the hydrophobic domain of pendolmycin is a C_5 allyl group and those of teleocidin A isomers are C_{10} linalyl groups, we thought that the tumor-promoting activity of pendolmycin would be weaker than those of the teleocidin A isomers. We (K.O., H.M. and M.N.) succeeded in totally synthesizing pendolmycin,[6] and this made it possible for us to investigate its biochemical, biological and tumor-promoting activities, and to compare them with those of the teleocidin A isomers.

In addition, 7-(nerolidyl)-(−)-indolactam-V, which has a C_{15} nerolidyl group attached to (−)-indolactam-V, was synthesized (Fig. 1). Thus, four compounds, which have differing lengths of hydrophobic domain on C-7, (−)- indolactam-V, pendolmycin, the teleocidin A isomers and 7-(nerolidyl)-(−)-indolactam-V are available to study the structure-activity relationships of the hydrophobic moiety of (−)-indolactam-V. Four activities, the inhibition of specific [^{3}H]TPA binding to a particulate fraction of mouse skin, activation of protein kinase C, induction of adhesion of HL-60 cells and induction of ornithine decarboxylase (ODC) in mouse skin were studied with these four compounds. This paper also reports a tumor-promoting activity of pendolmycin, which was weaker than that of the teleocidin A isomers and stronger than that of (−)-indolactam-V.[5]

MATERIALS AND METHODS

Materials Pendolmycin, (−)-indolactam-V and 7-(nerolidyl)-(−)-indolactam-V were chemically synthesized, as described previously.[6,7] Teleocidin A, which is a mixture of two isomers, was isolated from

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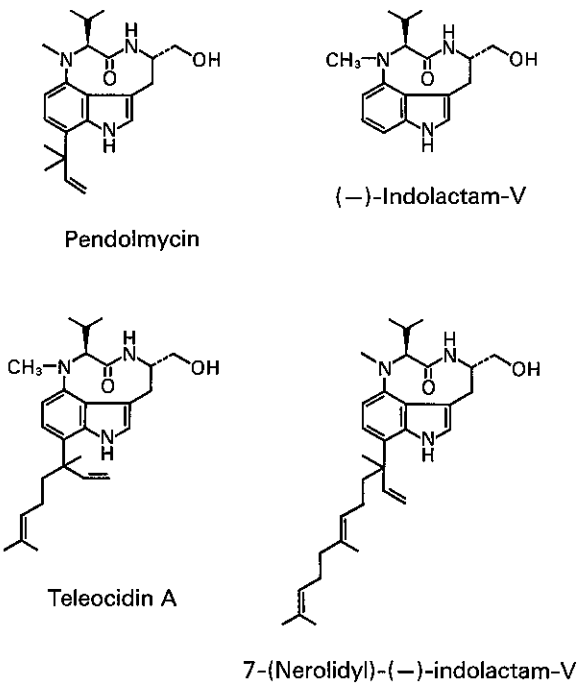

Fig. 1. Structures of pendolmycin and derivatives of teleocidin A.

Streptomyces mediocidicus.[2] [20-^{3}H(N)]TPA (specific activity 20 Ci/mmol) was purchased from New England Nuclear, Boston, MA. TPA was obtained from LC Services Corporation, Woburn, MA. DMBA was from Sigma Chemical Co., St. Louis, MO. Histone H1 was purchased from Boehringer Mannheim GmbH, FRG.

Inhibition of specific [^{3}H]TPA binding Specific [^{3}H]-TPA binding to a particulate fraction of mouse skin was measured by the cold-acetone filter method.[8] For the inhibition test, 100 μg of protein contained in a particulate fraction was incubated with 4 n*M* [^{3}H]TPA and a test compound in 1 ml of 20 m*M* Tris-HCl, pH 7.4, containing 2 m*M* 2-mercaptoethanol at 0°C for 2 h. Non-specific binding was measured in the presence of 500-fold excess of unlabeled TPA. Each value was determined by duplicate assay.

Activation of protein kinase C activity Protein kinase C was partially purified from mouse brain, as described previously.[9] Activity of protein kinase C was measured in terms of incorporation of ^{32}P into 50 μg of histone H1. The reaction mixture (250 μl) was incubated with 20 m*M* Tris-HCl (pH 7.5), 5 m*M* magnesium acetate, 30 μ*M* calcium chloride, 6.25 μg of phosphatidylserine, protein kinase C, a test compound and 125 μ*M* [^{32}P]ATP, at 30°C for 3 min. Each value was determined by duplicate assay.

Induction of HL-60 cell adhesion HL-60 cells were used at an initial density of 4×10^5 cells/ml of RPMI-1640 medium. Cell adhesion was determined 48 h after treatment with a test compound, as described previously.[10] Each value was determined by duplicate assay.

Induction of ODC in mouse skin ODC activity was determined by a method previously described.[10] A test compound dissolved in 0.2 ml of acetone was applied to depilated dorsal skin of an 8-week-old female CD-1 mouse. An epidermal extract was prepared to determine ODC activity 4 h after application of a compound. Each value was determined by duplicate assay.

Two-stage carcinogenesis experiment in mouse skin Carcinogenesis was initiated with a single application of 100 μg of DMBA, dissolved in 100 μl of acetone, to the skin of the backs of 8-week-old female CD-1 mice. From 1 week after initiation, 5 μg of pendolmycin, dissolved in 100 μl of acetone was applied to the same area of the skin up to week 15 of tumor promotion, twice a week, and from week 16 to week 30, 10 μg of pendolmycin was applied twice a week. As a positive control, 2.5 μg of teleocidin A was applied twice a week until week 30. The control groups were treated with pendolmycin alone or DMBA alone. Each group consisted of 15 mice.

RESULTS

Inhibition of specific [^{3}H]TPA binding Pendolmycin dose-dependently inhibited the specific [^{3}H]TPA binding to a particulate fraction of mouse skin (Fig. 2). The inhibitory effect of pendolmycin was stronger than that of (−)-indolactam-V and weaker than those of teleocidin A and 7-(nerolidyl)-(−)-indolactam-V. The ED_{50}

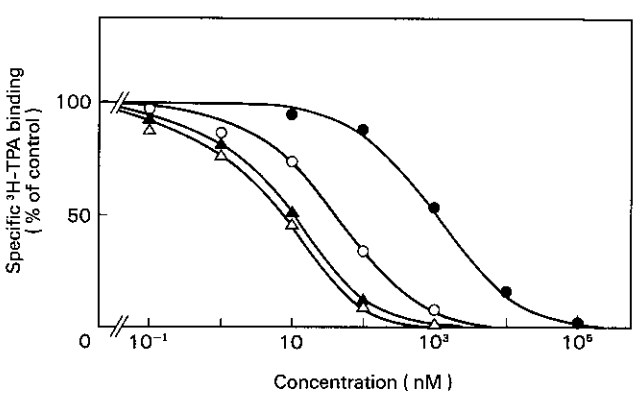

Fig. 2. Inhibition of specific [^{3}H]TPA binding to a particulate fraction of mouse skin by (−)-indolactam-V (●), pendolmycin (○), teleocidin A (▲) and 7-(nerolidyl)-(−)-indolactam-V (△). Each point represents the mean of duplicate determinations.

Table I. Biochemical, Biological and Tumor-promoting Activities of Pendolmycin and Derivatives of Teleocidin A

	Inhibition of specific [^{3}H]TPA binding[a] ED_{50} (nM)	Activation of protein kinase C[a] (cpm/100 nM compound)	Adhesion of HL-60 cells[a] ED_{50} (ng/ml)	Induction of ODC[a] (nmol CO_2 /mg protein /5 μg compound)	Tumor incidence at week 30 (%)
(−)-Indolactam-V	1,000	3,800	28	0.43	29[b]
Pendolmycin	37	7,200	7.5	1.6	73
Teleocidin A	9.6	9,200	2.7	3.5	100
7-(Nerolidyl)-(−)-indolactam-V	6.8	9,900	1.8	ND	ND

a) Each value was determined by duplicate assay.
b) Fujiki *et al.* (1985).[5]
ND: not determined.

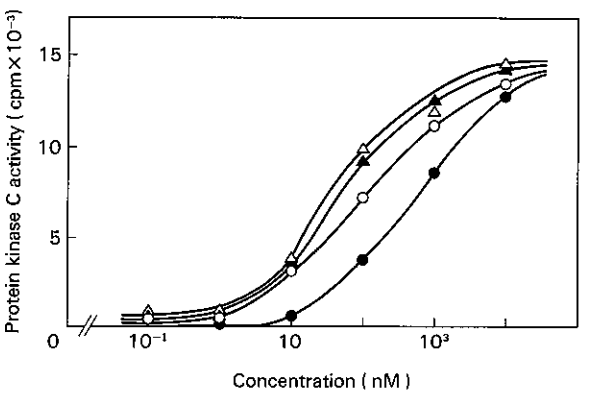

Fig. 3. Activation of protein kinase C partially purified from mouse brain by (−)-indolactam-V (●), pendolmycin (○), teleocidin A (▲) and 7-(nerolidyl)-(−)-indolactam-V (△). Each point represents the mean of duplicate determinations.

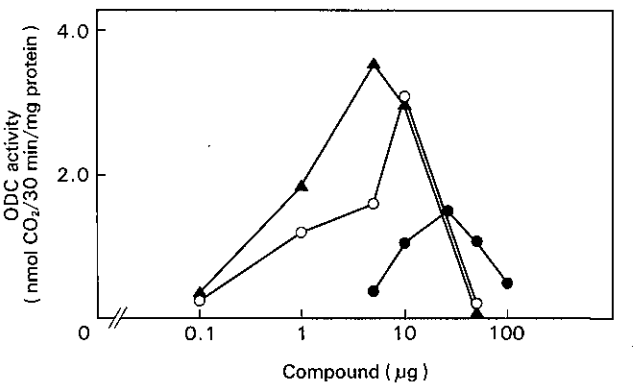

Fig. 4. Induction of ODC in mouse skin by (−)-indolactam-V (●), pendolmycin (○) and teleocidin A (▲). Each point represents the mean of duplicate determinations.

values, the concentrations of compounds which inhibited 50% of the specific [^{3}H]TPA binding, are summarized in Table I.

Activation of protein kinase C activity Pendolmycin dose-dependently activated protein kinase C activity (Fig. 3). The potency of pendolmycin for the activation was stronger than that of (−)-indolactam-V. The order of their potencies was well correlated with that of their binding affinities to the phorbol ester receptors (Table I).

Induction of HL-60 cell adhesion Pendolmycin induced adhesion of HL-60 cells to flasks. The ED_{50} values were 28 ng/ml for (−)-indolactam-V, 7.5 ng/ml for pendolmycin, 2.7 ng/ml for teleocidin A and 1.8 ng/ml for 7-(nerolidyl)-(−)-indolactam-V (Table I).

Induction of ODC in mouse skin Pendolmycin induced ODC activity more strongly than (−)-indolactam-V and not as strongly as teleocidin A. The maximum ODC activity induced by pendolmycin was 3.05 nmol CO_2/mg protein/30 min incubation, 4 h after application of the compound. The doses which induced the maximum activity were 25 μg for (−)-indolactam-V, 10 μg for pendolmycin and 5 μg for teleocidin A (Fig. 4).

Two-stage carcinogenesis experiment on mouse skin From the biochemical and biological activities of pendolmycin, which are shown in Table I, the tumor-promoting activity of pendolmycin was assumed to be weaker than that of teleocidin A. After initiation of the skin of mice with DMBA, 5 μg of pendolmycin was applied to the backs of mice, twice a week. The percentage of tumor-bearing mice in the group treated with DMBA plus pendolmycin was 6.5% in week 15. Application of an increased amount of the test compound increased the percentage gradually to 73.3% in week 25.

The group treated with DMBA plus teleocidin A yielded 100% tumor-bearing mice and 3.5 average number of tumors per mouse in week 28. The tumors

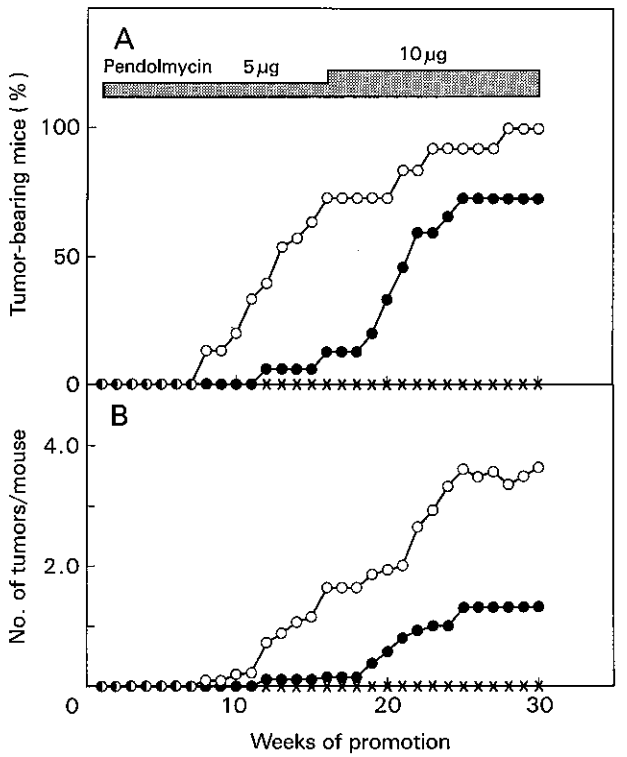

Fig. 5. Tumor-promoting activity of pendolmycin on mouse skin initiated with DMBA. (A) percentage of tumor-bearing mice, (B) average numbers of tumors per mouse. Groups were treated with DMBA plus pendolmycin (●), DMBA plus teleocidin A (○) and pendolmycin alone (×). Each group consisted of 15 mice. Data for the group treated with DMBA alone, which did not produce any tumors, are not shown.

appeared to be papillomas, but were not histologically studied. The groups treated with pendolmycin alone (Fig. 5) and DMBA alone (data not shown) did not develop any tumors.

DISCUSSION

A new class of tumor promoters, the teleocidin class, provides significant information concerning the mechanism of action of the TPA-type tumor promoters. The tumor-promoting activities of two teleocidin A isomers and four teleocidin B isomers are as potent as that of TPA, although they are structurally unrelated to TPA. The only structural difference between the teleocidin A and B isomers lies in the linalyl group in teleocidin A isomers and in the alkylated cyclohexene ring in teleocidin B isomers.[2] The (−)-indolactam-V portion is identical. Our previous study on the isomers of indolactam-V demonstrated that the 9S, 12S configuration of (−)-indolactam-V is necessary for biological activity, because (+)-indolactam-V and two isomers of *epi*-indolactam-V showed no activity.[4] On the other hand, N-1 substitution of a racemic mixture of indolactam-V with prenyl or geranyl groups did not enhance the activity significantly,[5] indicating that the hydrophobic moiety of teleocidin A should be attached to C-7 of (−)-indolactam-V, not to N-1, for maximum activity.

In this paper we report the structure-activity relationships of derivatives of the teleocidin A class, focusing on the role of the hydrophobic moiety attached to C-7 of the (−)-indolactam-V molecule: (−)-indolactam-V and pendolmycin can be considered as truncated derivatives of teleocidin A, while 7-(nerolidyl)-(−)-indolactam-V can be considered as a homologue with one additional isoprene unit. The first three compounds are naturally occurring, whereas the last one is synthetic. Their potency for binding to the phorbol ester receptors, activation of protein kinase C, induction of HL-60 cell adhesion and ODC induction in mouse skin increased depending on the length of the hydrophobic moiety. The potencies of the above four activities were well correlated with tumor-promoting activities for (−)-indolactam-V, pendolmycin, and teleocidin A.[5]

The interaction of teleocidin A with the phorbol ester receptors was investigated in a molecular modeling study using three-dimensional computer graphics. This provided a receptor cavity model for the TPA-type tumor promoters.[11] This model indicated that in addition to a hydrogen acceptor and two hydrogen donors, a tumor promoter should have a large hydrophobic moiety for activity. Although the interaction of the large hydrophobic moiety with some molecule in the receptor cavity model is not well clarified yet, biochemically, we assume that the hydrophobic moiety interacts with the lipid membrane, which is involved in the activation of protein kinase C, because a photoreactive probe for TPA covalently bound to phosphatidylserine and phosphatidylethanolamine.[12, 13] As shown in Fig. 3 and Table I, activation of protein kinase C was dramatically stimulated, with increase in the chain length of the terpenoid portion. Enhancement of the activity caused by C-7 substitution seems to reach a maximum with a C_{10} group, because the activity of 7-(nerolidyl)-(−)-indolactam-V was almost identical to that of teleocidin A. Although tumor-promoting activity of 7-(nerolidyl)-(−)-indolactam-V has not been tested yet on mouse skin because of its limited availability, the structure-activity studies with (−)-indolactam-V, pendolmycin and teleocidin A clearly indicated that a hydrophobic moiety attached to (−)-indolactam-V requires a reasonable volume, not only a suitable length, to fit into the receptor cavity and to induce maximum activity. 7-(Nerolidyl)-(−)-indolactam-V is indicated to be as potent a tumor promoter as teleocidin A.

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REFERENCES

1) Yamashita, T., Imoto, M., Isshiki, K., Sawa, T., Naganawa, H., Kurasawa, S., Zhu, B-Q. and Umezawa, K. Isolation of a new indole alkaloid, pendolmycin, from *Nocardiopsis*. *J. Nat. Prod.*, **51**, 1184–1187 (1988).
2) Fujiki, H., Suganuma, M., Ninomiya, M., Yoshizawa, S., Yamashita, K., Takayama, S., Hitotsuyanagi, Y., Sakai, S., Shudo, K. and Sugimura, T. Similar, potent tumor promoting activity of all isomers of teleocidins A and B in a two-stage carcinogenesis experiment on the skin of CD-1 mice. *Cancer Res.*, **48**, 4211–4214 (1988).
3) Umezawa, K., Imoto, M., Yamashita, T., Sawa, T. and Takeuchi, T. Modification of cellular membrane functions by pendolmycin. *Jpn. J. Cancer Res.*, **80**, 15–18 (1989).
4) Fujiki, H., Suganuma, M., Nakayasu, M., Tahira, T., Endo, Y., Shudo, K. and Sugimura, T. Structure-activity studies on synthetic analogues (indolactams) of the tumor promoter teleocidin. *Gann*, **75**, 866–870 (1984).
5) Fujiki, H., Suganuma, M., Hakii, H., Nakayasu, M., Endo, Y., Shudo, K., Irie, K., Koshimizu, K. and Sugimura, T. Tumor promoting activities of new synthetic analogues of teleocidin. *Proc. Jpn. Acad.*, **61**, 45–47 (1985).
6) Okabe, K., Muratake, H. and Natsume, M. Total synthesis of indole alkaloid pendolmycin. *Tetrahedron*, **46**, 5113–5120 (1990).
7) Endo, Y., Sudo, K., Itai, A., Hasegawa, M. and Sakai, S. Synthesis and stereochemistry of indolactam-V, an active fragment of teleocidins. Structural requirements for tumor-promoting activity. *Tetrahedron*, **42**, 5905–5924 (1986).
8) Ashendel, C. L. and Boutwell, R. K. Direct measurement of specific binding of highly lipophilic phorbol diester to mouse epidermal membranes using cold acetone. *Biochem. Biophys. Res. Commun.*, **99**, 543–549 (1981).
9) Kikkawa, U., Takai, Y., Minakuchi, R., Inohara, S. and Nishizuka, Y. Calcium-activated, phospholipid-dependent protein kinase from rat brain. *J. Biol. Chem.*, **257**, 13341–13348 (1982).
10) Fujiki, H., Mori, M., Nakayasu, M., Terada, M., Sugimura, T. and Moore, R. E. Indole alkaloids: dihydroteleocidin B, teleocidin, and lyngbyatoxin A as members of a new class of tumor promoters. *Proc. Natl. Acad. Sci. USA*, **78**, 3872–3876 (1981).
11) Itai, A., Kato, Y., Tomioka, N., Iitaka, Y., Endo, Y., Hasegawa, M., Shudo, K., Fujiki, H. and Sakai, S. A receptor model for tumor promoters: rational superposition of teleocidins and phorbol esters. *Proc. Natl. Acad. Sci. USA*, **85**, 3688–3692 (1988).
12) Delclos, K. B., Yeh, E. and Blumberg, P. M. Specific labelling of mouse brain membrane phospholipids with [20-^{3}H]phorbol-12-p-azidobenzoate 13-benzoate, a photolabile phorbol ester. *Proc. Natl. Acad. Sci. USA*, **80**, 3054–3058 (1983).
13) Schmidt, R., Heck, K., Sorg, B. and Hecker, E. Phospholipids involved in specific binding of 12-*O*-(5-azido-2-nitrobenzoyl)phorbol-13-acetate to epidermal microsomes, a photolabeling study. *Cancer Lett.*, **26**, 97–111 (1985).